GLP-I(7-36) Amide Augments Ba^{2+} Current Through L-Type Ca^{2+} Channel of Rat Pancreatic β-Cell in a cAMP-Dependent Manner

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The whole-cell patch-clamp method was used to examine the effect of glucagon-like peptide I (GLP-I)(7–36) amide on the activation process of L-type Ca^{2+} channels of rat pancreatic β-cells. After depolarization, GLP-I (1–100 nmol/l) caused action potentials in cells exposed to a glucose-free solution for 10 min. The percentage of cells producing action potential depended on the concentration of GLP-I. In some cells, GLP-I caused action potentials without the prior depolarization of the membrane. In cells exposed to the glucose-free solution for longer than 30 min, or in cells that were deprived of ATP by a means of the conventional whole-cell configuration, GLP-I (20 nmol/l) did not cause the electrical excitation. Application of GLP-I augmented the maximum Ba^{2+} current (I_{Ba}) through L-type Ca^{2+} channels and shifted the current voltage curve to the left. Values of changes in the maximum I_{Ba} depended on GLP-I concentration. Application of dibutyryl cAMP (dbcAMP, 1 mmol/l) also augmented I_{Ba}. In cells pretreated with Rp-cAMP, dbcAMP did not change the magnitude of I_{Ba}. Also in cells pretreated with Rp-cAMP, GLP-I failed to augment I_{Ba}. These results suggest that in pancreatic β-cells, GLP-I, by a cAMP-dependent mechanism, increases opening of L-type Ca^{2+} channels. cAMP-dependent augmentation of Ca^{2+} entry as well as cAMP production itself by GLP-I plays a crucial role in controlling insulin secretion. *Diabetes* 46:1755–1760, 1997

Glucagon-like peptide I (GLP-I)(7–36) amide as well as GLP-I(7–37) are potent physiological secretagogues of insulin from pancreatic islets (1–4). GLP-I functions through activation of a receptor resulting in formation of cAMP (5–8). The elevation of cAMP concentration and subsequent activation of protein kinase A (PKA) leads to insulin secretion as well as activation of insulin synthesis (9). It is known that GLP-I causes elevation of Ca^{2+} concentration ($[Ca^{2+}]_i$) by potentiating Ca^{2+} influx in the presence of extracellular glucose (12–14). Increase in $[Ca^{2+}]_i$ is known to mediate insulin secretion from pancreatic β-cells (15). Various mechanisms by which GLP-I accelerates the Ca^{2+} influx have been proposed: *1*) depolarization of the membrane leading to the opening of voltage-gated Ca^{2+} channels (14), *2*) opening of Ca^{2+}-permeable nonselective channels (16), and *3*) modulation of voltage-gated Ca^{2+} channels (17).

At least two types of voltage-dependent Ca^{2+} channels are known to be present in pancreatic β-cells (18–20), with the L-type Ca^{2+} channel predominantly important for controlling insulin secretion (21). The previous observation (17) indicates that GLP-I prolongs inactivation of the L-type Ca^{2+} channel by the cAMP-dependent mechanism (17). This fact is supported by the finding that application of membrane-permeable cAMP analogs prolonged the inactivation process (22), and this may be the mechanism for the augmentation by GLP-I of Ca^{2+} entry.

Here we provide another mechanism by which GLP-I augments Ca^{2+} entry into pancreatic β-cells through L-type Ca^{2+} channels: GLP-I, by a cAMP-dependent mechanism, potentiates the activation of L-type Ca^{2+} channel opening. cAMP-dependent regulation of the L-type Ca^{2+} channel has been shown in other cell types, including cardiac muscles (23–27), skeletal muscles (28), smooth muscles (29–31), and kidney connecting tubules (32). The potentiation of the activation process of Ca^{2+} channel opening can result not only in augmenting the Ca^{2+} entry but also in initiating Ca^{2+}-dependent action potential firing even without conditioning depolarization of the membrane. In fact, in the present study, GLP-I caused action potential firing in some cells without showing a very clear effect on the membrane potential before development of the action potentials. Both potentiation of the activation process and suppression of the inactivation process of the L-type Ca^{2+} channel seem to be involved in the GLP-I–dependent increase in Ca^{2+} entry in pancreatic β-cells.

RESEARCH DESIGN AND METHODS

Cell isolation. Isolation of islets was carried out as previously described (33). In brief, after being anesthetized with ether, the rats were bled by cutting the jugular vein. After occlusion of the common hepatic duct at the point close to the outlet mouth, a 10-ml Hank's solution containing collagenase (200 U/ml, Wako, Japan) was injected into the duct, which led to swelling of the pancreas with the enzyme solution. The pancreas was then taken out and moved into a plastic culture bottle and incubated for 20 min. After agitation by shaking the bottle, the suspension was filtrated with 0.5-mm metal mesh and washed with a solution with 2% albumin (bovine serum albumin [BSA]). About 200 islets were obtained from one rat. Islets were then cultured for 24 h with 5% CO_2. Separation of islet cells was performed using dispase (1,000 U/ml, Godo Shusei, Japan), as previously described (34). After washing cells with the solution containing 2% BSA, the suspension was moved into a culture medium. Cells were again cultured for 1–4 days. For experiments, cells were kept in a 35-mm Petri dish, and the dish was placed on an inverted microscope (IMT-2, Olympus, Japan). Only single cells were used

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BSA, bovine serum albumin; GLP-I, glucagon-like peptide I; GTP, guanosine 5′-triphosphate; PKA, protein kinase A.

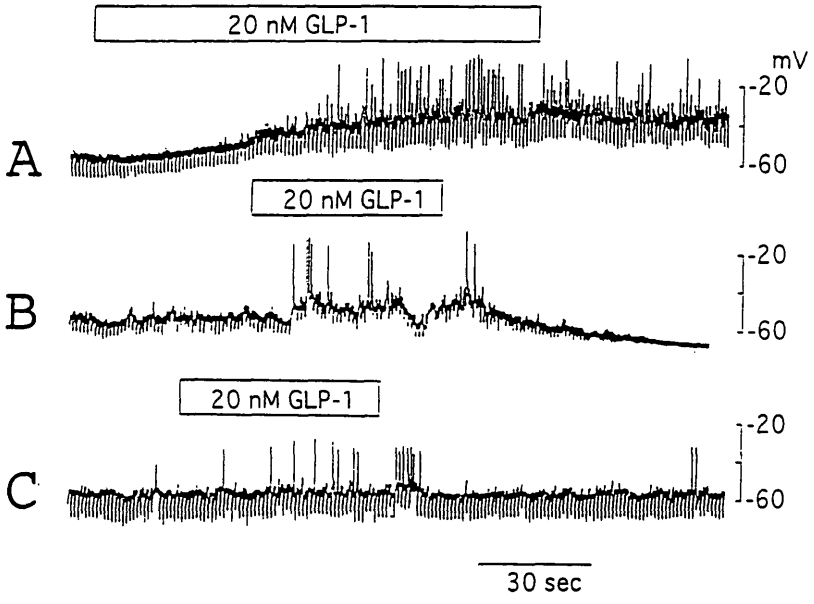

FIG. 1. Membrane potential responses of pancreatic β-cells to GLP-I. Membrane potentials of single pancreatic β-cells were recorded under the nystatin-perforated whole-cell configuration. Cells were exposed to a glucose-free solution for 10 min and then stimulated with GLP-I (20 nmol/l). Constant current pulses at 1 nA were applied throughout the experiments at 1 Hz. Three types of potential responses are shown. *A*: action potentials after depolarization of the membrane associated with a decrease in the membrane conductance. *B*: action potentials after depolarization of the membrane associated with an increase in the membrane conductance. *C*: action potentials after little change in the membrane potential.

for experiments. Identification of β-cells was carried out by detecting the excitatory responses of the cells to 15 mmol/l glucose or 1 mmol/l tolbutamide.

Electrical recordings. The whole-cell patch-clamp method (35) was used. Borosilicate glass capillaries (Dramonte) had resistances of 2–4 MΩ. For recording membrane potential, the nystatin-perforated whole-cell configuration (36,37) was used. Membrane currents were recorded with an EPC-7 patch-clamp amplifier (List Electronic, Germany). Membrane capacitance, measured by cancellation of the capacity transient, ranged from 11 to 18 pF. The average series resistance was 13.2 ± 3.1 MΩ(n = 12). Neither the cell capacitance nor the series resistance was electronically compensated during the experiments. The membrane potential was held at –60 mV, and 10-mV depolarizing steps (200 ms in duration) up to +30 mV and final step to +60 mV were applied by using a step pulse generator (SET-1201, Nihon Kohden, Japan). Currents evoked by the same sized voltage pulses gradually increased in magnitude and became constant after several trials. Drugs were applied to cells at a steady state. Leak subtraction was carried out by adding currents in response to inverted voltage pulses. The data were analyzed by using a whole-cell current analysis program (QP-120J, Nihon Kohden, Japan). All experiments were carried out at room temperature (around 22°C).

Solutions and drugs. The standard extracellular (bath) solution contained the following (in mmol/l): 105 NaCl, 5.6 KCl, 20 $BaCl_2$, 1.2 $MgCl_2$, 10 HEPES, and 5 TEA-Cl. The solution was adjusted to pH 7.2 with NaOH. In potential recordings, the bath solution contained $CaCl_2$ (1 mmol/l) instead of $BaCl_2$. A standard intracellular (pipette) solution contained the following (in mmol/l): 130 CsCl, 5 TEA-Cl, 4 $MgCl_2$, 10 HEPES, 4 Na_2ATP, 1 guanosine 5′-triphosphate (GTP), and 10 EGTA. The solution was kept at pH 7.2. In experiments using nystatin-perforated whole-cell potential recordings, the pipette solution contained only 135 mmol/l KCl and nystatin (200 μg/ml). The following drugs were used: GLP-I(7–36) amide (Peninsula), tolbutamide, nifedipine, N^6,2′-o-dibutyryladenosine 3′:5′-cyclic monophosphate (dbcAMP) (Sigma), and Rp-cAMP (Biolog, Germany). Cells in the experimental bath were superfused with a stream of an extracellular solution. For the drug application, drugs were dissolved in the extracellular solution, and the flowing solution was switched to one of these solutions.

Statistical analysis. Data were expressed as means ± SE of several experiments, and statistical significance was evaluated by using the Student's t test and Fisher's exact probability test. A value of $P < 0.05$ was accepted as significant.

RESULTS

Effects of GLP-I on the membrane potential of pancreatic β-cells. After exposure to a glucose-free solution for 10–15 min, 59 of 89 β-cells were electrically quiescent, measured under the nystatin-perforated whole-cell configuration. The resting membrane potentials of quiescent cells ranged from –33 to –68 mV (–48.3 ± 1.2 mV, n = 59). In 9 out of 16 quiescent cells examined, application of GLP-I (20 nmol/l) caused action potentials. Four cells showed action potentials after depolarization of the membrane associated with an increase in the membrane resistance (Fig. 1*A*). In two cells, action potentials followed membrane depolarization associated with a decrease in the membrane resistance (Fig. 1*B*). In three cells, action potentials appeared without a clear precedent change in the membrane potential (Fig. 1*C*). The percentage of the cells responding to GLP-I depended on the

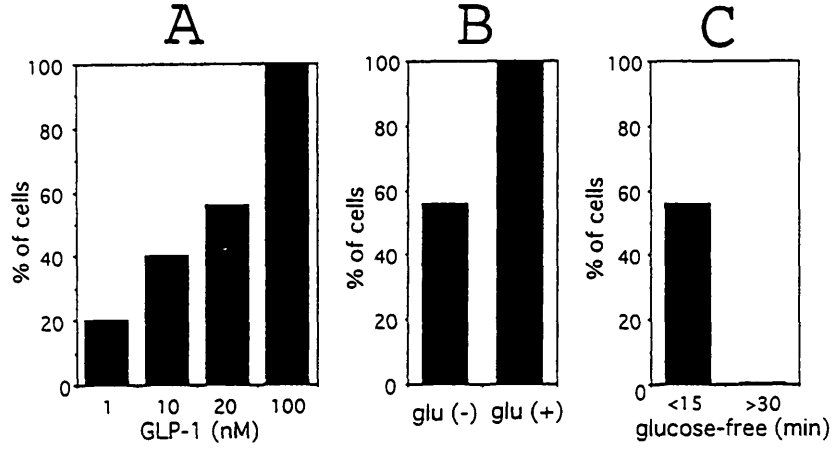

FIG. 2. Electrical excitation evoked by GLP-I under various conditions. *A*: concentration-response relationships. After a 10-min exposure to a glucose-free solution, GLP-I at different concentrations was applied. Percentages of cells showing action potentials in response to GLP-I are shown. Data at each concentration of GLP-I from 10 to 16 cells are shown. Difference between values at 1 and 20 nmol/l GLP-I is significant. *B*: in nine cells quiescent after exposure to 10 mmol/l glucose, 20 nmol/l GLP-I was applied. The data with the glucose-free solution are the same as that shown in *A*. Difference between two values is significant. *C*: in six cells that were exposed to the glucose-free solution for longer than 30 min, 20 nmol/l GLP-I was applied. The data with the glucose-free solution are the same as that shown in *A*. Difference between two values is significant.

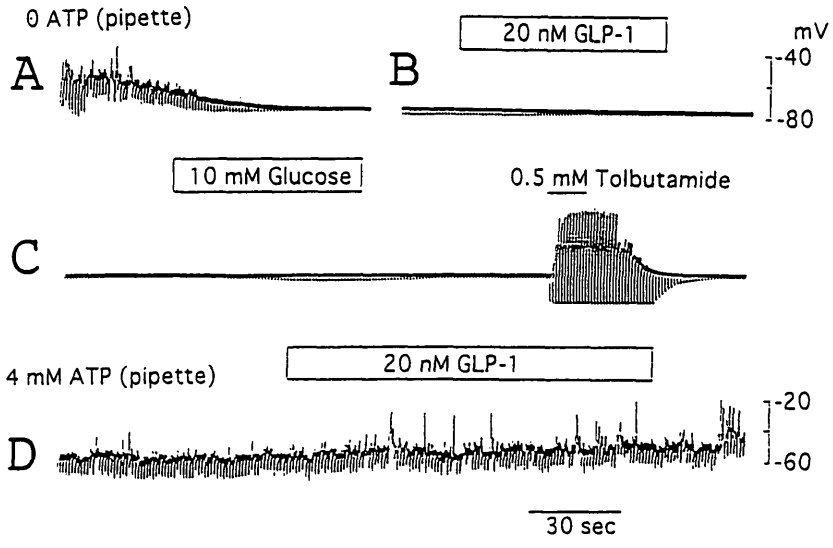

FIG. 3. GLP-I requires intracellular ATP for generation of action potentials. The conventional whole-cell configuration was made without ATP in the pipette solution. Traces in *A*, *B*, and *C* were taken from the same cell. *A*: time course of membrane potential and membrane conductance after exposure to the glucose-free solution. Constant current pulses were at 1 nA. *B*: GLP-I (20 nmol/l) stimulation after a 10-min exposure to the glucose-free solution. Constant current pulses were at 5 nA. *C*: glucose (10 mmol/l) stimulation caused a small depolarization. Tolbutamide (0.5 mmol/l) caused action potentials and a decrease in the membrane conductance. Constant pulses were at 5 nA. *D*: the whole-cell configuration with ATP (5 mmol/l) in the pipette solution. GLP-I (20 nmol/l) stimulation after a 10-min exposure to the glucose-free solution. Constant current pulses were at 1 nA. Similar results were obtained in eight other cells.

concentration of GLP-I (Fig. 2*A*). After exposure to 10 mmol/l glucose for 10 min, 9 out of 17 cells were still electrically quiescent. All of these cells were excited by application of GLP-I (20 nmol/l) (Fig. 2*B*). Cells exposed to the glucose-free solution for longer than 30 min did not respond to GLP-I (20 nmol/l) (Fig. 2*C*).

When the conventional whole-cell configuration was established with the pipette solution containing neither glucose nor ATP, the membrane potential became –72.1 ± 4.7 mV (n = 11) after exposure to the glucose-free solution (Fig. 3*A*). Under this condition, GLP-I (20 nmol/l) did not cause any change in membrane potential (Fig. 3*B*). Although glucose application caused only a small increase in membrane resistance, application of tolbutamide caused action potentials after depolarization (Fig. 3*C*). In the presence of 4 mmol/l ATP in the pipette solution, the resting membrane potential was similar to that observed with the nystatin method, and GLP-I caused action potentials (Fig. 3*D*).

Effects of GLP-I on the Ba^{2+} current of pancreatic β-cells. In 91 successful cells, the voltage showing the maximum inward current ranged from 0 to +20 mV. The magnitude of the maximum inward current also varied from cell to cell, ranging from 80 to 260 pA. The inward current was larger when Ba^{2+} was present in the extracellular solution than when it was absent. Nifedipine (10 μmol/l) or cadmium ion (50 μmol/l) markedly suppressed the inward current (not

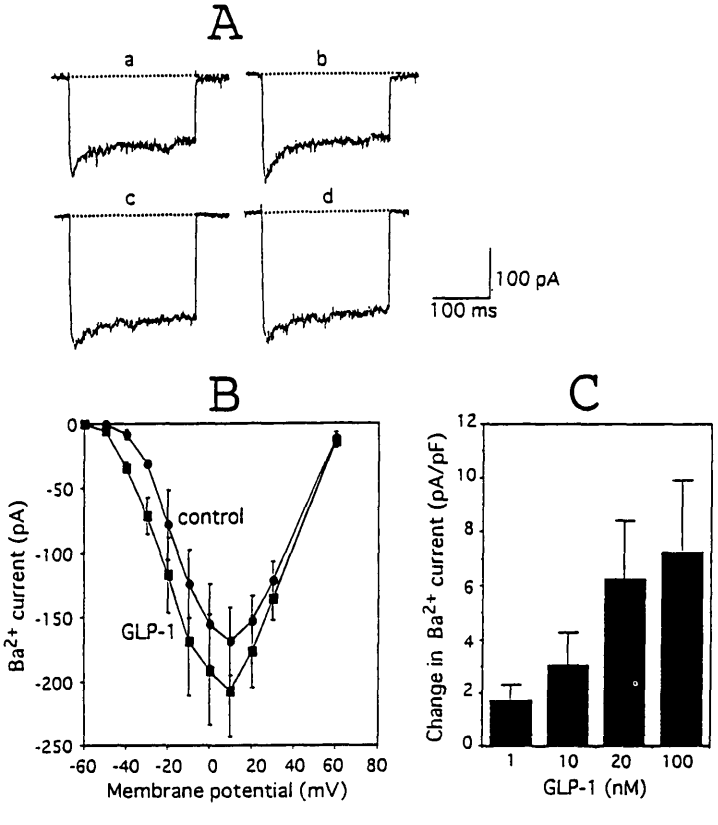

FIG. 4. Effects of GLP-I on the Ba^{2+} current through L-type Ca^{2+} channels. *A*: representative time course of the effect of GLP-I on the Ba^{2+} current (I_{Ba}). The maximum I_{Ba} caused by voltage steps to +10 mV before (*a*, *b*) and after (*c*, *d*) application of GLP-I (20 nmol/l) were shown. Intervals of each trace were 2 min. *B*: current-voltage relationships before (control) and after application of GLP-I (20 nmol/l). Values are the mean ± SE (vertical bars) from nine cells. Values of membrane potentials showing a half of the maximum current before and after application of GLP-I were –18 ± 2 and –23 ± 2 mV, respectively (P < 0.05). *C*: concentration-response relationships for GLP-I. Changes in the maximum I_{Ba} (expressed as current densities, pA/pF) were shown. The value at each concentration of GLP-I was taken from five to six experiments. Difference between values at 1 and 20 nmol/l GLP-I is significant.

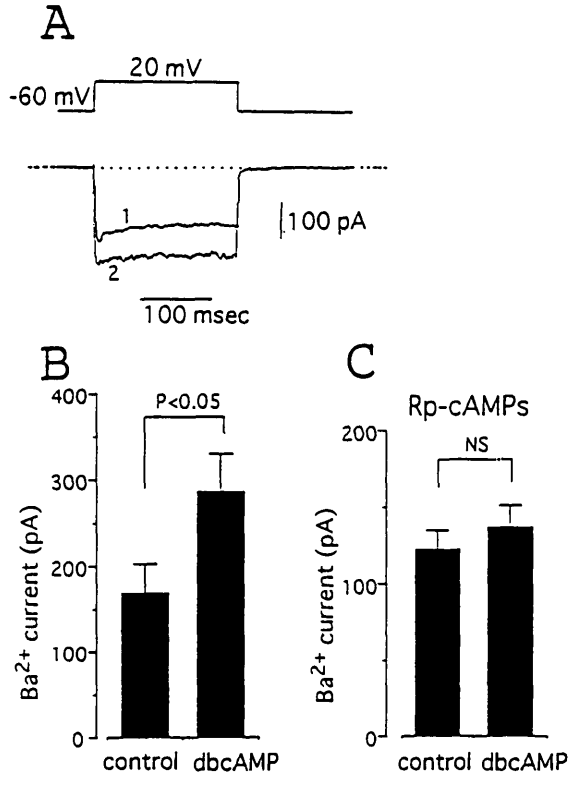

FIG. 5. Effects of dbcAMP on I_{Ba}. *A*: superimposed current traces showing the maximum I_{Ba} before (1) and after (2) application of 1 mmol/l dbcAMP. *B*: the mean values of the maximum I_{Ba} before and after application of dbcAMP (1 mmol/l) obtained from five cells. *C*: the mean values of the maximum I_{Ba} before and after application of dbcAMP (1 mmol/l) obtained from six cells pretreated with Rp-cAMP (10 mmol/l) for 30 min. Difference between two values is not significant.

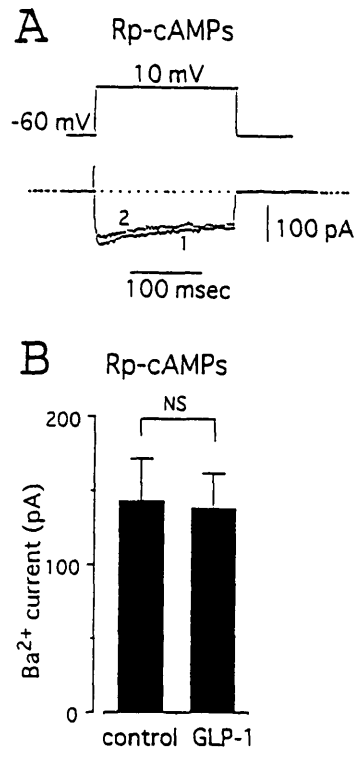

FIG. 6. Rp-cAMP inhibition of the GLP-I action on I_{Ba}. *A*: superimposed traces showing the maximum I_{Ba} before (1) and after (2) application of GLP-I (20 nmol/l) in the cell pretreated with Rp-cAMP (10 mmol/l) for 30 min. *B*: the mean values of maximum I_{Ba} before and after application of GLP-I (20 nmol/l) obtained from seven cells treated with 10 mmol/l Rp-cAMP. Difference between two values is not significant.

shown). Together, these results indicate that the current was carried by Ba^{2+} (Ba^{2+} current, I_{Ba}) through L-type Ca^{2+} channels. GLP-I (20 nmol/l) caused an increase in magnitude of I_{Ba} (Fig. 4*A* and *B*). In 31 cells tested, the mean values of the maximum I_{Ba} were 121 ± 16 and 158 ± 18 pA ($P < 0.05$) before and after application of GLP-I (20 nmol/l), respectively. This effect of GLP-I lasted for longer than 10 min (Fig. 4*A*). Net changes of the maximum I_{Ba}/pF in response to GLP-I depended on the concentration of GLP-I (Fig. 4*C*). The increase in I_{Ba} caused by GLP-I was observed at all voltages examined (Fig. 4*B*), with the potential for half maximum current shifted to a more negative value (Fig. 4*B*).

I_{Ba} was increased also by application of dbcAMP (1 mmol/l) (Fig. 5*A* and *B*). In cells pretreated with Rp-cAMP (10 μmol/l), dbcAMP (1 mmol/l) failed to augment I_{Ba} (Fig. 5*C*). In cells pretreated with Rp-cAMP (10 μmol/l), GLP-I (20 nmol/l) also failed to increase I_{Ba} (Fig. 6*A* and *B*).

DISCUSSION

GLP-I causes action potentials of pancreatic β-cells dependent on intracellular ATP. It is widely accepted that the action of GLP-I on the β-cell function depends on extracellular glucose (4,12–14,38). This proposal was confirmed by the present study because GLP-I at 20 nmol/l excited all cells in the presence of glucose (10 mmol/l), while it excited only 56% of cells exposed to a glucose-free solution for <15 min (Fig. 2*C*). As also shown in Fig. 1*A–C*, GLP-I caused action potentials in β-cells even in the absence of glucose in the extracellular solution. However, GLP-I did not excite any cell that was exposed to the glucose-free solution for longer than 30 min (Fig. 2*C*). On the other hand, in cells exposed to the glucose-free solution for longer than 30 min, GLP-I still generated action potentials when the pipette solution contained 4 mmol/l ATP (Fig. 3*D*). These findings together indicate that the excitatory action of GLP-I on the membrane potential requires the presence of ATP in the cell and that glucose is important for maintaining the level of ATP.

GLP-I activation of Ba^{2+} current (I_{Ba}) through L-type Ca^{2+} channels. The data shown in Fig. 4 and the block of I_{Ba} by nifedipine suggest that GLP-I increases current through L-type Ca^{2+} channels. In addition, GLP-I shifted the I–V curves to the left (Fig. 4*B*). This effect on the activation of the Ca^{2+} current by GLP-I explains the fact that GLP-I caused action potentials even without a very clear change in the membrane potential before development of the action potential firing (Fig. 1*C*). Because the augmentation of I_{Ba} by GLP-I was canceled by application of Rp-cAMP, an inhibitor of PKA (17,22,39,40) (Fig. 6), it is clear that GLP-I activated I_{Ba} via the activation of PKA, resulting from an elevation of cAMP. GLP-I receptors are present in the islets of Langerhans (8,41), and the activation of β-cells by GLP-I is mediated by GTP-binding proteins (Gs) (7,42,43). In fact, it has been shown that GLP-I increases the concentration of cAMP in the pancreatic β-cells (5–8).

There is evidence that cAMP and agents that raise the cytosolic cAMP level clearly augment glucose-dependent islet cell bursting (44). In recent studies, however, a cAMP-dependent mechanism has been shown to have little or no

effect on the amplitude of the voltage-gated Ca^{2+} current in mouse and rat islet cells (17,22,45), although this mechanism clearly slowed the time course of decay of the Ca^{2+} current (17, 22) in mouse pancreatic β-cells. The principal action of cAMP on the Ca^{2+} current was, therefore, considered to be slowing the inactivation rather than increasing the maximal conductance (22). Prolonged inactivation of I_{Ba} by GLP-I (probably by cAMP) was also observed in the present study (Fig. 4*A*). On the other hand, in the present study on rat islet β-cells, the magnitude of I_{Ba} through L-type Ca^{2+} channels was increased by application of dbcAMP (Fig. 5). The difference between results obtained in the previous experiments (17,22) and those in the present experiment may be because of the difference of species (mice in the previous studies, rats in the present study) from which pancreatic β-cells were taken. Another possible explanation is that the characteristics of the Ca^{2+} channels examined in the previous and present studies were different because of differences in experimental conditions. The Ca^{2+} channel protein enters several conformational states, resulting in changes in its voltage dependency and the kinetics of the channel opening (25). This possibility is supported by the observation that I_{Ba} showed run-up with time after establishment of the whole-cell configuration.

In conclusion, GLP-I activates L-type Ca^{2+} channels in pancreatic β-cells in a cAMP-dependent manner by potentiating the activation process and suppressing the inactivation process of the channel opening. Such cAMP-dependent regulation by GLP-I of the Ca^{2+} channel as well as elevation of the cAMP level itself (22,45) seem to play an important role in controlling insulin secretion from pancreatic β-cells.

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